

Influence of egg size on egg and larval development of *Sympetrum striolatum* at different prey availability (Odonata: Libellulidae)

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ABSTRACT

Egg size differences might have an important influence on reproductive success because they may lead to different offspring conditions, hatching date or larval size. We presumed that egg size in odonates positively correlates with egg development time, and larger eggs lead to larger larvae. However, we assumed that the size benefit could only be maintained under harsh, but not under good conditions. Harsh and good conditions were simulated by different diets with specific feeding intervals; high prey level fed every day, low prey level fed every second day. The prey organisms used were *Artemia salina* and *Chironomus riparius*. The study was conducted with the libellulid *Sympetrum striolatum*. Our results showed that larger eggs caused a longer development time. Larger eggs resulted in significantly larger first instar larvae. However, larger larvae maintained their size benefit only in the high prey level with *C. riparius*. We found no significant differences between low prey and high prey level within the two prey types. We therefore assume that the differences between the two prey levels in this study were not large enough. In general, *A. salina* seems to be more nutritious than *C. riparius* for the first larval stadia.

INTRODUCTION

Life history theory is based on the existence of trade-offs among traits that contribute to reproductive success (Stearns 1992; Roff 2000). Thereby, clutch size, egg size, egg development time, and larval size are some of the parameters that influence reproductive success. They can be influenced by maternal effects (Corkum et al. 1997; Mousseau & Fox 1998), since females with different conditions have different resources to allocate to their offspring (Hanes 1992). As reproductive success is expected to be maximised, a trade-off between offspring number (i.e. clutch

size) and offspring size is assumed (Smith & Fretwell 1974; Kagata & Ohgushi 2004). Egg size is presumed to be correlated with the amount of resources for embryo development, egg development time, early instar stadia growth, and larval size, and therefore exerts a strong influence on offspring condition (Johnston & Leggett 2002). Variation in egg size can be found within different populations (Stearns 1992; Johnston & Leggett 2002) or even within egg clutches (Einum & Fleming 2004; Schenk & Söndgerath 2005) of a single species.

A crucial assumption is that offspring condition and survival increase with egg size (Tessier & Consolatti 1991; Gillooly & Dodson 2000). Thus, the production of large eggs, for instance, provides the offspring hatching from these eggs with better conditions (Santo et al. 2001). Egg development time usually decreases exponentially with egg size in aquatic insects (Gillooly & Dodson 2000). Corkum et al. (1997) showed that offspring hatching from larger eggs were larger than offspring from smaller ones. Thereby, larger larvae are in a better condition to avoid predators (Fleming & Gross 1990). Cannibalism and intraguild predation are highly represented in Odonata with larger individuals feeding on smaller ones (Johnson 1991; Anholt 1994; Hopper et al. 1996; Padeffke & Suhling 2003).

Sibling rivalry (Johnson et al. 1985) is a consequence of the high number of offspring, asynchronous hatching time, and different body sizes. Even slight time and size differences within a single egg clutch become important. Longer egg development time can be either an advantage or a disadvantage. Anholt (1994) showed that the later hatching larvae are more influenced by cannibalism, but if the time window is large enough, the older larvae would already be large enough so that the following larvae are then too small to serve as prey items. First hatching larvae might be in the position to find more nutritious prey, because they are larger and have a greater possibility to feed on larger prey than smaller larvae do. In later stadia, however, maternal effects and egg size are often less important than environmental conditions (Sweeney 1984; Sebens 1987) with type, quality, and quantity of prey resources. Therefore, it is unclear, whether large early stadium larvae can maintain their size benefit from maternal size and egg size during larval development. It is of interest, whether these effects can be enlarged or simulated under different prey conditions. There is evidence that maternal and egg size benefits diminish with progressing larval development under good conditions (Schenk & Söndgerath 2005). However, it is possible that these effects may have a greater influence under harsh conditions.

In our study we investigated three hypotheses: (1) egg size correlates positively with egg development time, (2) larger larvae hatch out of larger eggs, and (3) prey quality, as well as prey quantity, influence the larval growth. Thereby, we expect larvae from larger eggs to be able to maintain a size benefit during larval development under harsh, but not under good, conditions. We raised larvae of *Sympetrum striolatum* (Charpentier) derived from eggs of different sizes under two different prey qualities and two different prey quantities. *Artemia salina* and *Chironomus riparius* were used as feeding organisms. As the chitin content of *C. riparius* is lower than of *A. salina* (Cauchie 2002) we expect *C. riparius* to be the more nutritious prey than *A. salina* for dragonfly larvae. For the two prey quantity treatments good conditions consisted of prey ad libitum and harsh conditions included enough prey to survive.

MATERIAL AND METHODS

Females of *Sympetrum striolatum* were caught on two lakes (52°17'N, 10°40'E; 52°14'N, 10°38'E) near Braunschweig, Lower Saxony. To prevent seasonal influences females were captured within a short period (8-16 August 2004). The females were caught immediately after copulation to make certain that the eggs were fertilised. Oviposition was initiated by dipping the females abdomen into jars filled with water (cf. Boehms 1971) and afterwards the head width was measured. Length and width of 64 randomly selected eggs per female from 10 individuals were measured using a dissecting microscope with an ocular micrometer to a precision of 0.05 mm (50x magnification). We calculated the egg circumference (C) using the formula: $C = \pi * (1.5 * (a+b) - \sqrt{a * b})$ with $a = 0.5 * \text{length}$ and $b = 0.5 * \text{width}$. After measuring the eggs, they were separated randomly into four different treatments (see below). The eggs were stored in 8 ml dechlorinated tap water under constant temperature (20 °C) and received 11 h of neon light per day. Larval hatching was monitored every day until 25 November 2004. Egg development time was recorded for each egg as the time from oviposition to the first free-living larval stadium. Head width of hatched larvae was measured as an index of body size (cf. Benke 1970) using a dissecting microscope with an ocular micrometer to a precision of 0.05 mm (50x magnification). Hatched larvae were transferred to bigger jars (height 27 mm) with 90 ml protozoan water, washed sand (0.1-0.4 mm), and eight blades of grass (40 mm length). The larvae were reared under identical conditions to the eggs.

The larvae were reared under four different treatments: (1) low prey level with *Artemia salina*, (2) high prey level with *A. salina*, (3) low prey level with *Chironomus riparius*, and (4) high prey level with *C. riparius*. "High prey level" treatments were fed every day and the "low prey level" every second day. The amounts of prey consisted of around an average of 15 *C. riparius* per larvae and 75 *A. salina* per larvae in each feeding solution. The head widths of the larvae were measured every 12 days. The larvae were reared until they passed the fifth measuring point.

Statistical analysis

Regression analyses were used to investigate correlations between egg size, egg development time, and the larval size. Thereby, we took egg circumference as factor versus egg development time and the head widths from the first up to the fifth larval head width measurement as dependent variable. In order to analyse the influence of the treatments on the larval size, we conducted a two-way ANCOVA with prey quality and quantity as factors, the female head width as a co-variable, and the larval head widths of the different measurements as the dependent variables. We excluded the first larval head measurement because this measurement was taken directly after hatching and so before the larvae were fed with different prey treatments.

Table 1. Results of all regression analyses for the influence of egg circumference on egg development time and larval size of *Sympetrum striolatum*.

Source	n	Slope	r ²	p
Egg circumference / egg development time	312	13.132	0.012	0.053
Egg circumference / first measurement	207	0.043	0.097	< 0.0001
Egg circumference / second measurement	175	0.217	0.065	< 0.0001
Egg circumference / third measurement	156	0.266	0.020	0.063
Egg circumference / fourth measurement	104	0.398	0.032	0.028
Egg circumference / fifth measurement	83	0.598	0.026	0.057

RESULTS

There was a trend that larger eggs required a longer egg development time (Table 1). Egg circumference also had a significant positive influence on the head widths of the first larval head measurement (Table 1). Additionally, egg size showed a significant influence on the second and fourth head width measurement, but only a trend at the third and fifth head width measurement (Table 1).

The quality and quantity of the prey fed exerted a significant influence on the head width of the larvae, whereas the maternal size did not (Table 2). The larval size differed depending on the feeding organism. In general larvae fed with *Artemia salina* grew larger than those fed with *Chironomus riparius* (Fig. 1). In the fourth and fifth larval measurements also the interaction term between prey quality and prey quantity was significant (Table 2). The Fisher post hoc test showed a significant difference between the treatments with different prey qualities, but no differences between the treatments of the two prey quantities within the same feeding organism (Table 3).

DISCUSSION

According to the results of Schenk & Söndgerath (2005) there is a trend for longer egg development time of larger eggs. Our study confirms that larger eggs lead to larger first instar larvae, as described by Corkum et al. (1997). There might be a trade off between egg development time and larval size. Smaller eggs seem to need a shorter egg development time but result in smaller larval size, whereas larger eggs result in larger larvae with a longer egg development time. Thereby, larvae that hatched earlier might be in a position to receive temporary and more nutritious prey because some prey organisms are rare, or occur only during a particular time period. However, conditions may be unfavourable when the first larvae hatch – for example, older, and therefore larger, larvae may already colonize the habitat. Larval size has an important influence, because even minimal larval size differences within a single egg clutch can lead to cannibalism (Anholt 1994). Odonate larvae are generalist predators that interact aggressively with con- or hetero-specifics

Table 2. Results of a two-way ANCOVA for *Sympetrum striolatum* with prey quality and quantity as factors, female head width as a co-variable and larval head width of the different larval stadia as dependent variables.

Variable	Source	F	d.f.	p
Second measurement	Prey quality	17.29	1	< 0.0001
	Prey quantity	6.26	1	0.014
	Interaction: quality x quantity	0.36	1	0.549
	Maternal size	0.01	1	0.936
Third measurement	Prey quality	66.56	1	< 0.0001
	Prey quantity	3.75	1	0.056
	Interaction: quality x quantity	2.73	1	0.102
	Maternal size	1.59	1	0.210
Fourth measurement	Prey quality	86.21	1	< 0.0001
	Prey quantity	11.18	1	0.001
	Interaction: quality x quantity	7.36	1	0.008
	Maternal size	2.05	1	0.155
Fifth measurement	Prey quality	94.23	1	< 0.0001
	Prey quantity	7.45	1	0.008
	Interaction: quality x quantity	4.82	1	0.031
	Maternal size	0.20	1	0.658

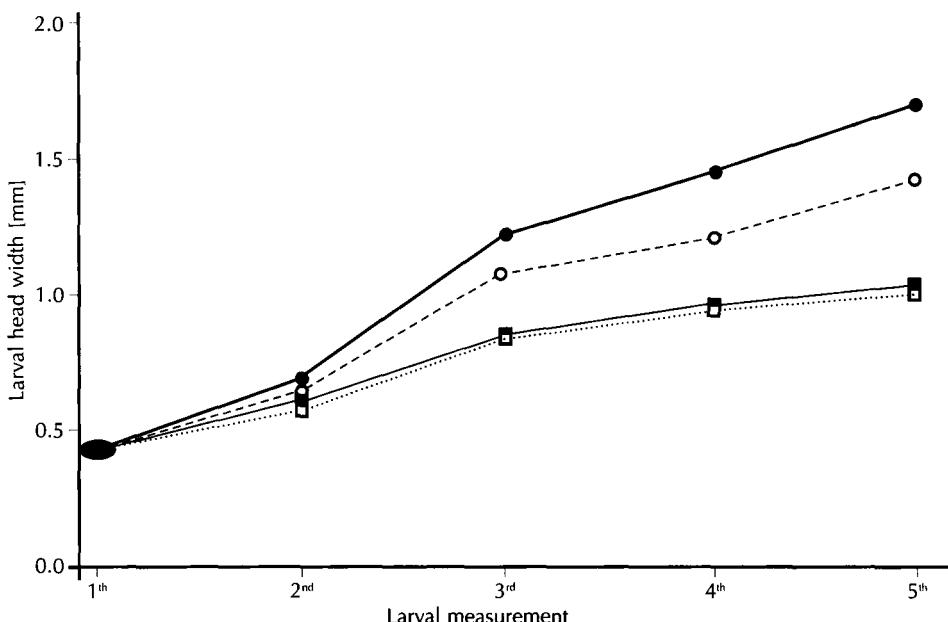


Figure 1: Influence of prey treatments on larval *Sympetrum striolatum* — comparison of the head width [mm] \pm s.d. of all four prey treatments; thick drawn through line: high *Artemia salina*; dashed line: low *A. salina*; thin drawn through line: high *Chironomus riparius*; dotted line: *C. riparius*.

(Rowe 1980; Baker 1981). Larger larvae generally feed on smaller larvae that might be particularly important in the early larval stadia (Anholt 1994; Hopper et al. 1996; Suhling & Lepkojus 2001; Padelfke & Suhling 2003). In summary, to produce a size range of eggs within each clutch (see Schenk et al. 2004; Schenk & Söndgerath 2005) might be seen as some kind of risk spreading.

One question is why did larger larvae hatch out of larger eggs, and why did larvae out of larger eggs hatch later? One possible explanation might be some additional vitellin. Insect egg vitellin consists of high molecular mass phospholipoglycoprotein (Hagedorn & Kunkel 1979) that has a nutritive function for the developing embryo (McGregor & Loughton 1974, 1977). Larger eggs with more vitellin might therefore have more energy and better starting conditions. Furthermore, the composition of other ingredients might vary with egg size. Potentially there could be specific salts, enzymes, hormones, or mineral nutrients that give embryos of larger eggs better starting conditions. In birds for example, the amount of carotenoids per egg is assumed to enhance immune function and hence, egg condition (Blount et al. 2002). Otherwise, egg yolk has a nutritious role for the growing embryo (Masuda & Oliveira 1985) and a higher amount of vitellin may give the embryo more resources to grow, and this may be the only difference between different sized eggs. Additionally, it is possible, that there are differences in body tissues of the embryos that cause different egg sizes. However, in a study on fishes, Einum et al. (2002) assumed that a higher amount of egg yolk in larger eggs is what makes the egg larger, and not the body tissue. To our knowledge, this has not been investigated in insects so far. Future studies might yield valuable and interesting insights.

In general, the study showed a significant difference between the two prey qualities; *Artemia salina* was a more nutritious prey for dragonfly larvae than *Chironomus riparius*. Larvae of *Sympetrum striolatum* that were fed on *C. riparius* did not grow as large as the larvae fed on *A. salina*. As the chitin content of *C. riparius* is lower than of *A. salina* (Cauchie 2002) the results opposed our expectations. However, it could be that even the young larvae of *C. riparius* were too large to be considered as prey for the relatively small larvae (Baker 1989). Nevertheless, for later larval stadia *C. riparius* should be the more nutritious prey. Other explanations might be the different behaviour and movement of the prey. In a free water body *C. riparius* sidles in S-curves but they often stayed at one point in the sand on the ground in a constructed case (Brönmark & Hansson 1998; Dathe 2003). This behaviour may help them to avoid predators. In contrast, *A. salina* moved directly through the water body most of the time. It could be easier for dragonflies to catch moving *A. salina* than the rather immobile *C. riparius* because dragonfly larvae are mainly visual hunters (Sherk 1977). Additionally, the density of prey differed between the two organisms. The density of *A. salina* was higher than of *C. riparius*, so the probability to find prey was lower for *C. riparius* than for *A. salina*. The morphology of the prey might also play a role. *C. riparius* is more serpentine and even later libellulid larval stadia may need more time and energy to swallow them. In contrast, *A. salina* is more compact and may be easier to swallow. Nevertheless, it is still uncertain if feeding with *A. salina* produces an artefact, as it is not a natural prey for Odonata larvae. The habitats of both species differ, *A. salina* live in salt water (Crome et al. 1969; Gruner et al. 2003) whereas libellulids live more or less

Table 3. Results of the Fisher post hoc test for the larvae of *Sympetrum striolatum* with the four different prey treatments and the larval head width for four measurements (*: $p < 0.05$).

Prey level	Second	Third	Forth	Fifth
High <i>A. salina</i> / high <i>C. riparius</i>	*	*	*	*
High <i>A. salina</i> / low <i>A. salina</i>	-	-	-	-
High <i>A. salina</i> / low <i>C. riparius</i>	*	*	*	*
High <i>C. riparius</i> / low <i>A. salina</i>	*	*	*	*
High <i>C. riparius</i> / low <i>C. riparius</i>	-	-	-	-
Low <i>A. salina</i> / low <i>C. riparius</i>	*	*	*	*

in fresh water (Corbet 1999). *A. salina* has, however, often been used successfully to raise dragonfly larvae (Suhling & Lepkojus 2001; Schenk & Söndgerath 2005; Suhling et al. 2005).

When larger larvae can maintain their egg size benefit for several larval stadia, they are in the position to feed on smaller larvae even within the same larval stadium (Anholt 1994; Padeffke & Suhling 2003). We presumed that large larvae could maintain the size benefit of larger eggs under harsh conditions. Harsh and good conditions were simulated by different diets. However, there were no significant differences between the two feeding levels within the same prey organisms. Lawton et al. (1980) found that the final three instars of *Ischnura elegans* (Vander Linden), fed with one *Daphnia magna* every third day, were able to survive for periods of up to 146 days. Therefore, we suggest that our low prey level still contained too much prey and so it has not simulated harsh conditions. Whether or not another feeding rate will solve this problem needs to be investigated.

Prey quality and quantity appeared to respond in the same way in the second and third larval measurement, as the interaction terms were not significant. In contrast, at the fourth and fifth larval measurement the interaction terms between prey quality and prey quantity were significant. This means that the varying larval sizes respond in different ways to prey quality and prey quantity. It would be nice to explain this by the fact that in earlier larval stadia the maternal effects might have a greater influence than environmental or prey conditions. However, even on the early larval stadia, we did not find a significant influence of maternal size on larval size of the early larval stadia. This is contrary to the results of several other studies, that found maternal effects to influence clutch size, egg size, larval size and larval growth, because females of different sizes have different amounts of resources to allocate to their offspring (Hanes 1992; Corkum et al. 1997; Mousseau & Fox 1998; Schenk & Söndgerath 2005; Koch & Suhling 2005).

Low prey and high prey levels did not differ significantly within the two prey types. It appears that all treatments simulated good conditions. Therefore, this study confirms the assumption that under good conditions the effects of maternal and egg size diminishes with progressing larval development (Sebens 1987; Schenk & Söndgerath 2005), excluding the high prey treatment with *C. riparius*. In later stadia other traits and environmental conditions become more important. However, we still cannot explain whether larvae from larger eggs might maintain their size benefit under harsh conditions.

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